

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

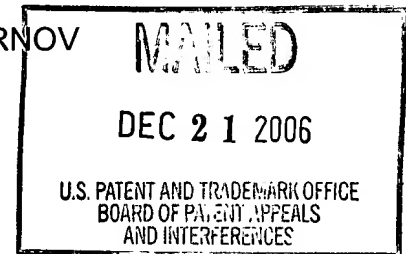
**UNITED STATES PATENT AND TRADEMARK OFFICE**

**BEFORE THE BOARD OF PATENT APPEALS  
AND INTERFERENCES**

Ex parte ALAN WOLFFE and FYODOR URNOV

Appeal No. 2006-2851  
Application No. 09/844,501

ON BRIEF



Before ADAMS, MILLS, and GRIMES, Administrative Patent Judges.

MILLS, Administrative Patent Judge.

**DECISION ON APPEAL**

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 123-152.

Claims 123 reads as follows:

123. A method for preparing a library of regulatory DNA sequences from a cell, the method comprising:

- (a) providing a cell nucleus, wherein the nucleus comprises cellular chromatin;
- (b) contacting the nucleus with a first enzyme, wherein the first enzyme reacts with accessible regions of cellular chromatin;
- (c) deproteinizing the cellular chromatin to generate deproteinized DNA;

Appeal No. 2006-2851  
Application No. 09/844,501

(d) contacting the deproteinized DNA with a second enzyme to generate DNA fragments;

(e) contacting the DNA fragments obtained in step (d) with a population of vector molecules, wherein the vector molecules comprise a first end that is compatible with the first enzyme and a second end that is compatible with the second enzyme, under conditions favorable to ligation of compatible ends; and

(f) selecting polynucleotides comprising a DNA fragment ligated to a vector molecule.

The prior art cited by the examiner is:

Li et al. (Li)	5,500,356	March 19, 1996
Grosveld et al. (Grosveld)	5,635,355	June 3, 1997
Chung	6,644,421	Sept. 3, 2002

NEB Catalog, pp. 32, 46, 48, and 83 (1995)

#### Grounds of Rejection

Claims 123-128, 130, 135, 143-145, and 147-151 stand rejected under 35 U.S.C. § 103(a) over Grosveld.

Claims 129, 131-133, and 152 stand rejected under 35 U.S.C. § 103(a) over Grosveld taken with Li.

Claims 136-142 stand rejected under 35 U.S.C. § 103(a) over Grosveld taken with NEB catalog (1995).

Claims 134 and 146 stand rejected under 35 U.S.C. § 103(a) over Grosveld taken with Chung.

We reverse these rejections.

DISCUSSION

35 U.S.C. § 103

Claims 123-128, 130, 135, 143-145, and 147-151 stand rejected under 35 U.S.C. § 103(a) over Grosveld.

According to the examiner Grosveld teaches each of the claimed steps, with particular reference to column 8, lines 1-25, column 15, lines 43-47 and column 21, lines 18-20 and claim 1 (Answer, pages 3-4).

Upon review of the disclosure of Grosveld, we do not find the examiner has provided sufficient evidence to support a prima facie case of obviousness of the method of claim 123.

We agree with the Examiner that Grosveld describes steps (a)-(d) of the method of claim 123 at Column 8, lines 1-25, we do not find that Grosveld describes a method consistent with steps (e)-(f) of the claimed method.

In particular, claim 123, step (e) recites, "contacting the DNA fragments obtained in step (d) with a population of vector molecules, wherein the vector molecules comprise a first end that is compatible with the first enzyme and a second end that is compatible with the second enzyme, under conditions favorable to ligation of compatible ends."

Grosveld at column 8, lines 16-32, describes deproteination steps and digestion with a second enzyme to generate fragments, such as BglII, consistent with steps (c)

and (d) of claim 123. Then, the “exact location of the DNaseI hypersensitive site[s] of the 3’ of the adult  $\beta$ -globin gene were determined using two single copy DNA probes and several restriction enzyme digests of DNaseI digested HEL nuclei. The data summarized in FIG. 2 (A-D) show that there is a single DNaseI hypersensitive site between the 2.3 kb BglIII fragment and the 2.4 kb HindIII fragment . . .” Column 8, lines 48-51. Accordingly, Grosveld obtained fragments of the adult  $\beta$ -globin gene and probed these fragments to locate the DNaseI hypersensitive site. Grosveld did not, according to claim 123, step (e), contact the DNA fragments obtained in step (d) with a population of vector molecules, wherein the vector molecules comprise a first end that is compatible with the first enzyme and a second end that is compatible with the second enzyme, under conditions favorable to ligation of compatible ends; or step (f), select polynucleotides comprising a DNA fragment ligated to a vector molecule. Grosveld, on the other hand, probed DNA fragments which were not ligated to a vector, and selected the DNA fragment of interest having the DNaseI hypersensitive site by its ability to bind to a probe.

In a different experiment, Grosveld incorporated the previously identified DNase I hypersensitive sites into a vector or plasmid containing both the hypersensitive sites and the adult  $\beta$ -globin gene. Column 15, lines 6-47. The DNA fragments cloned in the experiment described in column 15 are not the same as the fragments described in column 8. In particular, the hypersensitive site (HSS)-containing fragments cloned in col. 15 are not the DNaseI restriction enzyme fragments from col. 8. See col. 15, lines

45-46: PvuI-BstEII fragment with HSS 1 and 2; BstEII-ClaI fragment with HSS 3 and 4.

In contrast, appellants describe their method in the specification, pages 49-50, as follows.

In another embodiment, cellular chromatin is subjected to limited nuclease action, and fragments having one end defined by nuclease cleavage are preferentially cloned. For example, isolated chromatin or permeabilized nuclei are exposed to low concentrations of DNase I, optionally for short periods of time (e.g., one minute) and/or at reduced temperature (e.g., lower than 37°C). DNase-treated chromatin is then deproteinized and the resulting DNA is digested to completion with a restriction enzyme, preferably one having a four-nucleotide recognition sequence. ... Preferential cloning of nuclease-generated fragments is accomplished by a number of methods. For example, prior to restriction enzyme digestion, nuclease-generated ends can be rendered blunt-ended by appropriate nuclease and/or polymerase treatment (e.g., T4 DNA polymerase plus the 4 dNTPs). Following restriction digestion, fragments are cloned into a vector that has been cleaved to generate a blunt end and an end that is compatible with that produced by the restriction enzyme used to digest the nuclease treated chromatin. ... Ligation of adapter oligonucleotides, to nuclease-generated ends and/or restriction enzyme-generated ends, can also be used to assist in the preferential cloning of fragments containing a nuclease-generated end. For example, a library of accessible sequences is obtained by selective cloning of fragments having one blunt end (corresponding to a site of nuclease action in an accessible region) and one cohesive end ...

In the method of claim 123, it is only after the DNA fragments have been ligated to a vector molecule that the polynucleotide of interest is selected. See, Example 15, specification, page 114, lines 6-14, wherein E.coli colonies harboring insert-containing plasmids were identified and screened.

While both appellants and the examiner rely heavily on argument with respect to potential limitations within the preamble the claims, we do not find it necessary to reach

Appeal No. 2006-2851  
Application No. 09/844,501

this issue to decide the case before us. For the reasons discussed herein, we do not find the examiner has provided sufficient evidence to support a prima facie case of obviousness. The rejection of the claims over Grosveld is reversed.

35 U.S.C. § 103(a)

Claims 129, 131-133 and 152 stand rejected under 35 U.S.C. § 103(a) over Grosveld taken with Li. Claims 136-142 stand rejected under 35 U.S.C. § 103(a) over Grosveld taken with NEB catalog (1995). Claims 134 and 146 stand rejected under 35 U.S.C. § 103(a) over Grosveld taken with Chung.

With respect to the other pending obviousness rejections before us, all rejections stand or fall on the relevance of Grosveld to the pending claims. The examiner relies on the NEB catalog to make up for a failure of Grosveld to teach specific restriction enzymes (Answer, page 6), Li for a failure of Grosveld to teach a comparison of cells from a variety of different sources (Answer, page 7), and Chung for the failure of Grosveld to teach embedding cells in agarose prior to enzymatic cleavage (Answer, page 9).

We do not find that either NEB catalog, Li or Chung overcome the above noted deficiency of Grosveld and its failure to teach steps (e) and (f) of claim 123, and therefore the rejections for obviousness over Grosveld taken with NEB catalog, Li or Chung are reversed.

Appeal No. 2006-2851  
Application No. 09/844,501

CONCLUSION

The rejections of the claims under 35 U.S.C. § 103(a) over Grosveld alone or in view of NEB, Li or Chung are reversed.

REVERSED



Donald E. Adams  
Administrative Patent Judge



Demetra J. Mills  
Administrative Patent Judge



Eric Grimes  
Administrative Patent Judge

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Appeal No. 2006-2851  
Application No. 09/844,501

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